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Monoamine Oxidase Inhibitors from a Lichen, *Solorina crocea* (L.) ACH.

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Three anthraquinones, solorinic acid, averantin 6-monomethyl ether and 4,4′-bissolorinic acid, and two depsides, methyl gyrophorate and gyrophoric acid were isolated from a lichen, *Solorina crocea* (L.) ACH. The monoamine oxidase inhibitory potencies were observed only in the anthraquinones.

Keywords——*Solorina crocea*; lichen; monoamine oxidase inhibitor; solorinic acid; averantin 6-monomethyl ether; 4,4′-bissolorinic acid; methyl gyrophorate; gyrophoric acid

During the assay of natural products having the monoamine oxidase (MAO) inhibitory effect in our laboratory, norsolorinic acid from *Emericella navahoensis* NFM 42019 was found to be a potent inhibitor of MAO *in vitro*.[1] Since this fungus produced only very limited amounts of norsolorinic acid and the related compounds, we tried to isolate them from a lichen, *Solorina crocea* (L.) ACH. which was known to contain anthraquinones, and the MAO inhibitory potencies of these anthraquinones were tested.

The lichen collected in Canada was extracted with acetone at 5°C for three days and then under reflux for 6 h to give acetone-exts. 1 and 2, respectively. The residue was refluxed in chloroform for further extraction to give $CHCl_3$-ext.

Crystallization of $CHCl_3$-ext. (12.7 g) from benzene yielded reddish orange needles of pig. 1 (8.6 g). The mother liquid was subjected to silica gel column chromatography. Fr. D eluted with *n*-hexane–benzene was further purified by preparative (prep.) TLC to obtain pig. 3 (4 mg). By flash chromatography (Chart 1) of the benzene-eluate (fr. H), pig. 2 (22 mg) was obtained.

The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of the major pigment, pig. 1, was similar to that of norsolorinic acid except that the former had the signal of methoxy methyl at δ 3.94. By the comparison of the spectral data, pig. 1 was considered to be solorinic acid which was known to be contained in this lichen[2] (Fig. 1). In the mass spectrum (MS) pig. 2 had the molecular ion of m/z 386, higher than that of solorinic acid by two protons. The ^{1}H-NMR spectrum of pig. 2 indicated the presence of a secondary alcohol moiety at δ5.45 (CH, ddd, $J=7.7$, ~4.0, 3.3 Hz) and 2.69 (OH, d, $J=3.3$ Hz), suggesting that pig. 2 has the structure of solorinic acid reduced at the side chain, that is, averantin 6-monomethyl ether.[3] The molecular ion of pig. 3 by MS was m/z 766, corresponding to twice that of solorinic acid minus two protons. In the ^{1}H-NMR spectrum of pig. 3, the signal of an aromatic methine at C-4 was not observed. The signal at δ7.09 assigned to C-5 proton was observed in a higher field than the corresponding signal of solorinic acid because of the anisotropy of anthraquinone-ring. From these data, the structure of pig. 3 was elucidated to be 4,4′-bissolorinic acid.[3]

Acetone-ext. 1 and 2 gave a similar TLC pattern. On TLC, crude acetone-ext. 1 showed several pigments, pig. 1 and 2, norsolorinic acid, averantin and averythrin, all identified by comparison with the authentic samples. The amounts of those pigments seemed to be very small, but acetone-ext. 1 were also contained a lot of colorless materials. Then, the colorless compounds were isolated from this extract as follows. Acetone-ext. 1 (14 g) was washed with chloroform and the insoluble residue (8.6 g) was dissolved in acetone to apply on a Sephadex LH-20 column (acetone). Crude compds. A (4.0 g)

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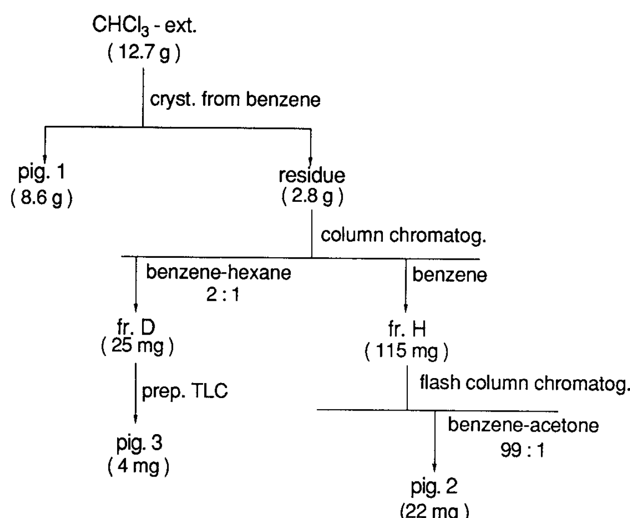
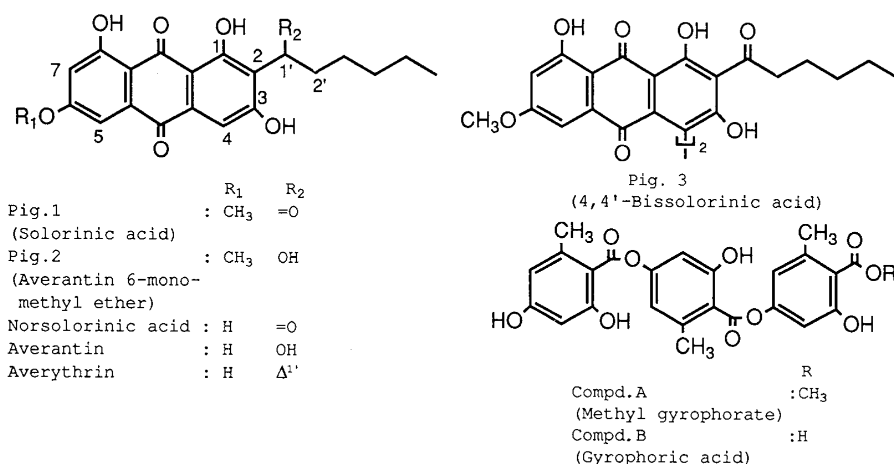


Chart 1. Isolation Procedure of $CHCl_3$-ext.



	R_1	R_2
Pig.1 (Solorinic acid)	CH_3	=O
Pig.2 (Averantin 6-mono-methyl ether)	CH_3	OH
Norsolorinic acid	H	=O
Averantin	H	OH
Averythrin	H	$\Delta^{1'}$

Fig. 1. Structures

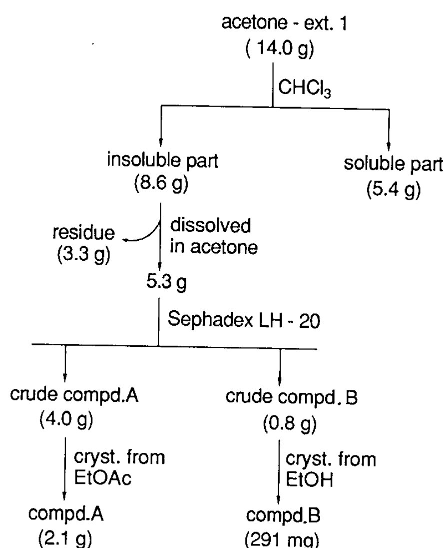


Chart 2. Isolation Procedure of Acetone-ext. 1

and B (0.8 g) obtained were crystallized from ethyl acetate and ethanol, respectively (Chart 2).

The ^{1}H-NMR spectrum of compd. A showed the presence of three aromatic methyl signals at δ2.59, 2.63 and 2.70 and three pairs of meta-coupled aromatic methine signals at δ6.31 and 6.33, 6.60 and 6.74,

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and 6.68 and 6.78. A methoxy methyl at δ3.99 and four hydroxyl signals at δ5.33, 11.08, 11.21 and 11.52 were also observed. It seemed that compd. A was a trimer made up of similar phenolic components. Hydrolysis of compd. A gave solely orsellinic acid. By the comparison of the NMR spectra and the TLC (Merck HPTLC; Kieselgel F_{254}, benzene : ethyl acetate 5/1; RP-18, methanol : water 5/1) of compd. A with those of the authentic methyl gyrophorate,[4] these two compounds were found to be identical. The mixed mp was not depressed.

The NMR spectrum of compd. B was similar to that of compd. A, except that no methoxy methyl was observed in compd. B. The hydrolysis of compd. B yielded orsellinic acid, as in the case of compd. A. Permethylated compd. B was identical with tetramethyl-compd. A. The molecular weight (468) of compd. B given by FAB-MS also supported that compd. B is gyrophoric acid.[5] This is the first isolation of gyrophoric acid from *S. crocea*.

MAO inhibitory potency was measured by the modified Kraml's method which was reported previously.[1] The anthraquinones, solorinic acid and averantin 6-monomethylether, exhibited the potencies of IC_{50} 14.3 and >100 μM, respectively. They were less potent than the corresponding desmethyl compounds, norsolorinic acid (IC_{50} 0.3 μM)[1] and averantin (IC_{50} 77.9 μM), respectively. The dimer, 4.4′-bissolorinic acid did not show any significant effect (IC_{50} >100 μM). The depsides, gyrophoric acid and methyl gyrophorate, had no effects. Studies on the roles of the functional groups of anthraquinones in the MAO inhibitory potency are in progress.

The latter two depsides were also tested for their HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibition, because the inhibitory effect of the mixture containing gyrophoric acid had been reported.[6] However, no significant effects were observed with the dose of up to 100 μg/ml.[7]

Experimental

Apparatus——Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. The spectral data were obtained on the following instruments; IR on Hitachi EPI-G3 and 260-10; UV on Hitachi U-3200; ^{1}H- and ^{13}C-NMR on JEOL GSX 400 and 500; EI-MS on Hitachi M-60; FAB-MS on Finnigan MAT TSQ-70.

Extraction and isolation——Dried lichen (1 kg) collected in Canada was kept in acetone at 5°C for 3 days. The acetone extract was evaporated to give acetone-ext. 1 (27.9 g). The residue was refluxed in acetone for 6 h and then in chloroform for 6 h (×3) to give acetone-ext. 2 (9.8 g) and $CHCl_3$-ext. (14.0 g), respectively. Pig. 1 (8.6 g) was obtained by crystallization of $CHCl_3$-ext. (12.7 g) from benzene. The residue (2.8 g) was subjected to silica gel column chromatography. Fr.D eluted with *n*-hexane–benzene 1/2 was purified by prep. TLC to yield pig. 3 (4 mg). Benzene-eluate, fr.H (115 mg) was flash chromatographed with benzene–acetone 99/1 to give pig. 2 (22 mg). Acetone-ext. 1 (14 g) was washed with chloroform, and the precipitates (8.6 g) were dissolved in acetone. After removing the insoluble residue, the soluble part (5.3 g) was separated on Sephadex LH-20 column chromatography to give crude compds. A (4.0 g) and B (0.8 g), which were further purified by crystallization.

Pig. 1 (solorinic acid)——orange needles from benzene, mp 206~7°C (lit.[2a] mp 203°C). UV λ_{max} ($CHCl_3$) nm (log ε): 271.5, 281 sh, 313, 367 sh, 463.5, 486 (4.33, 4.31, 4.43, 3.56, 4.02, 3.97). IR ν_{max} (KBr) cm^{-1}: 3425, 1680, 1633, 1622, 1586, 1390, 1309, 1261, 1171. ^{1}H-NMR δ($CHCl_3$): 0.94 (3H, t-like, J=7.0), 1.37—1.43 (4H, m), 1.71—1.77 (2H, m), 3.24 (2H, t, J=7.4), 3.94 (3H, s), 6.71 (1H, d, J=2.5), 7.33 (1H, s), 7.36 (1H, d, J=2.5), 12.16 (1H, s), 14.38 (1H, s), 14.39 (1H, s).

Pig. 2 (averantin 6-monomethyl ether)——orange needles from benzene, mp 209–210°C (lit.[3] mp 213°C). EI-MS m/z (%): 386 (M^+, 1.3), 368 (50), 339 (21), 325 (48), 315 (17), 311 (100), 299 (48). UV λ_{max} ($CHCl_3$) nm (log ε): 251.5 sh, 266, 291.5, 319, 448, 467 sh (4.12, 4.19, 4.41, 3.96, 4.01, 3.98). IR ν_{max} (KBr) cm^{-1}: 3445, 1665, 1613, 1598, 1445, 1394, 1252, 1207. ^{1}H-NMR δ($CHCl_3$): 0.90 (3H, t-like, J=7.0), 1.30—1.39 (5H, m), 1.49–1.56 (1H, m), 1.79–1.94 (2H, m), 2.69 (1H, d, J=3.3), 3.92 (3H, s), 5.45 (1H, ddd, J=7.7, ~4.0, 3.3), 6.66 (1H, d, J=2.6), 7.27 (1H, s), 7.34 (1H, d, J=2.6), 9.62 (1H, s), 12.26 (1H, s), 12.71 (1H, s).

Pig. 3 (4,4′-bissolorinic acid)——reddish ppt from benzene. EI-MS m/z (%): 766 (M^+, 100), 748 (7), 695 (9), 677 (8), 384 (13), 366 (6), 341 (20), 328 (10), 313 (55). UV λ_{max} ($CHCl_3$) nm: 273.5, 320.5, 375 sh, 484.5, 508 sh. ^{1}H-NMR δ($CHCl_3$): 0.94 (3H, t-like, J=7.0), 1.39–1.42 (4H, m), 1.74–1.78 (2H, m), 3.31 (2H, dd, J=8.1, 6.2), 3.84 (3H, s), 6.68 (1H, d, J=2.5), 7.09 (1H, d, J=2.5), 12.17 (1H, s), 14.96 (1H, s), 15.08 (1H, s); each H×2.

Compound A (methyl gyrophorate)——white granules from ethyl acetate, mp 283–284.5°C (dec.) (lit.[4] mp 288°C). EI-MS m/z (%): 182 (40), 150 (100), 122 (47), 94 (14). UV λ_{max} (ethanol) nm (log ε): 213.5, 270, 305 (4.82, 4.44, 4.23). IR ν_{max} (KBr) cm^{-1}: 3465, 1679, 1612, 1584, 1418, 1308, 1252, 1200, 1148, 1073. ^{1}H-NMR δ($CHCl_3$, 40°C): 2.59 (3H, s), 2.63 (3H, s), 2.70 (3H, s), 3.99 (3H, s), 5.33 (1H, br. s), 6.31 (1H, d, J=2.3), 6.33 (1H, d, J=2.3), 6.60 (1H, d, J=2.4), 6.68 (1H, d, J=2.2), 6.74 (1H, d, J=2.4), 6.78 (1H, d, J=2.2), 11.08 (1H, s), 11.21 (1H,

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s), 11.52 (1H, s).

Compound B (gyrophoric acid)——white granules from ethanol, mp 258–266°C (dec.) (lit.[5] mp 220°C). FAB-MS *m/z*: 469 $(M+H)^+$. UV λ_{max} (ethanol) nm (log ε): 213, 271, 305 (4.84, 4.43, 4.24). IR ν_{max} (KBr) cm^{-1}: 3300, ~3000 br, 1662, 1602, 1457, 1307, 1240, 1203, 1143, 1068. ^{1}H-NMR δ(DMSO-d_6): 2.38 (6H, s), 2.40 (3H, s), 6.24 (1H, s), 6.25 (1H, s), 6.62 (1H, d, J=1.3), 6.65 (1H, s), 6.67 (1H, d, J=1.3), 6.69 (1H, s), 10.04 (1H, br. s), 10.37 (1H, s), 10.50 (1H, s). ^{13}C-NMR δ(DMSO-d_6): 19.4 (CH_3), 20.1 (CH_3), 21.5 (CH_3), 100.5 (CH), 107.3 (CH, ×2), 108.1 (C), 110.0 (CH), 114.3 (CH), 114.6 (CH), 116.7 (C), 118.0 (C), 138.0 (C), 139.7 (C) 140.4 (C), 152.1 (C), 152.3 (C), 156.4 (C), 159.0 (C), 160.3 (C), 161.3 (C), 165.7 (C), 167.3 (C), 170.6 (C).

Methylation of compound A——Diazomethane in ether was added to the THF solution (2.5 ml) of compd. A (63 mg). The mixture was stirred at room temperature for 4.5 h, and then, a small amount of acetic acid was added. After evaporation of the solvent the residue was separated by prep. TLC (Merck Kieselgel 60 F_{254}; toluene: ethyl acetate : acetic acid 20/15/1). Di-, tri- and tetra-methyl derivatives were obtained (11, 18 and 4 mg, respectively). Dimethyl-compd. A, ^{1}H-NMR δ(acetone-d_6): 2.46 (3H, s), 2.57 (3H, s), 2.66 (3H, s), 3.88 (3H, s), 3.96 (3H, s), 4.00 (3H, s), 6.41 (1H, d, J=2.5), 6.47 (1H, dd, J=2.6, 0.7), 6.73 (1H, dd, J=2.3, 0.7), 6.76 (1H, d, J=2.2), 6.90 (1H, d, J=2.0), 7.06 (1H, d, J=1.9), 11.25 (2H, br. s), Trimethyl-compd. A, ^{1}H-NMR δ(acetone-d_6): 2.30 (3H, s), 2.46 (3H, s), 2.66 (3H, s), 3.86 (6H, s), 3.88 (3H, s), 3.96 (3H, s), 6.41 (1H, d, J=2.7), 6.47 (1H, dd, J=2.5, 0.8), 6.80 (1H, d, J=1.9), 6.83 (1H, d, J=1.9), 6.90 (1H, d, J=1.9), 7.05 (1H, d, J=1.7), 11.23 (1H, br. s). Tetramethyl-compd. A, ^{1}H-NMR δ(acetone-d_6): 2.30 (3H, s), 2.40 (3H, s), 2.45 (3H, s), 3.86 (9H, s), 3.91 (3H, s), 3.95 (3H, s), 6.50 (1H, d, J=~2.0), 6.54 (1H, d, J=2.2), 6.79 (1H, dd, J=1.9, 0.6), 6.82 (1H, d, J=1.9), 6.83 (1H, dd, J=1.9, 0.8), 6.87 (1H, d, J=1.9).

Methylation of compound B——An ether solution of diazomethane was added to compd. B (13 mg) in THF (0.5 ml). The mixture was stirred at room temperature for 69 h, and then a small amount of acetic acid was added. Purification of the reaction mixture by preparative TLC gave tetra- and penta-methylated compd. Bs (2 and 4 mg, respectively).

Hydrolysis of compound A——Compd. A (63 mg) in 3 ml of 32 N-H_2SO_4 was stirred at 0°C for 2 h. After the addition of ice, the reaction mixture was extracted with ether. Orsellinic acid (41 mg) was purified by Sephadex LH-20 column chromatography eluted with acetone. Orsellinic acid, colorless needles from acetone-water, mp 177–9°C. UV λ_{max} (ethanol) nm (log ε): 214.5, 260, 298.5 (4.42, 4.13, 3.67). IR ν_{max} (KBr) cm^{-1}: 3365, ~3000 br, 1643, 1620, 1460, 1363, 1265, 1172. ^{1}H-NMR δ(acetone-d_6): 2.52 (3H, s), 6.23 (1H, dd, J=2.5, 0.6), 6.29 (1H, dd, J=2.5, 0.8), 9.11 (1H, br. s), 12.14 (1H, br. s). ^{13}C-NMR δ(acetone-d_6): 24.8 (CH_3), 102.0 (CH), 105.3 (C), 112.6 (CH), 145.4 (C), 163.8 (C), 167.7 (C), 174.7 (C).

Hydrolysis of compound B——Compd. B (14 mg) in 1.5 ml of 32 N-H_2SO_4 was stirred at 0°C for 2.5 h. Extraction with ether followed by purification gave orsellinic acid (12 mg).

Preparation of crude MAO——Male ddY mice obtained from Shizuoka Agricultural Cooperative Association (Hamamatsu, Japan) were used. Each mouse liver was homogenized with 4 volumes of 0.15 M-KCl in a Teflon homogenizer under ice cooling. The homogenates were centrifuged at 1000×g for 10 min. The supernatants were used for the assay.

Assay of MAO activity——The MAO activity was assayed by the modified Kraml's method[1,8] with kynuramin as a substrate. Samples dissolved in DMSO were added to the incubation medium (final concentration of DMSO: 2.8%). By fluorometry at 380 nm with excitation at 315 nm, 4-hydroxyquinoline which was formed from kynuramin by MAO was measured.

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